PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01N 33/367, A61K 49/00, 43/00 — A1

(11) International Publication Number: WO 94/28412

(43) International Publication Date: 8 December 1994 (08.12.94)

(21) International Application Number: PCT/US94/05809

(22) International Filing Date: 27 May 1994 (27.05.94)

(30) Priority Data:
08/069,010 28 May 1993 (28.05.93) US

(71) Applicant: THE MIRIAM HOSPITAL [US/US]; 164 Summit Avenue, Providence, RI 02099 (US).

(72) Inventors: MAROTTA, Charles, A.; 1 Richdale Avenue, #8, Cambridge, MA 02140 (US). MAJOCHA, Ronald, E.; 288 West Street, Needham, MA 02194 (US).

(74) Agents: SAXE, Bernhard, D. et al.; Foley & Lardner, Suite 500, 3000 K Street, N.W., Washington, DC 20007-5109 (US).

(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, SD, SE, SK, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: COMPOSITION AND METHOD FOR *IN VIVO* IMAGING OF AMYLOID DEPOSITS

(57) Abstract

An amyloid binding composition for *in vivo* imaging of amyloid deposits comprising a labeled amyloid protein or variant thereof which binds to amyloid deposits *in vivo*; and a pharmaceutically acceptable carrier, is described. Methods of detecting amyloid deposits and for diagnosing Alzheimer's Disease and Down's Syndrome are also described.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

COMPOSITION AND METHOD FOR IN VIVO IMAGING OF AMYLOID DEPOSITS

Background of the Invention

The present invention relates to the identification of compositions which are suitable for use in *in vivo* imaging of amyloid deposits and methods related thereto. More specifically, the present invention relates to a method of diagnosing Alzheimer's Disease.

Alzheimer's Disease ("AD") is the most common cause of dementia in the United States, and the presence of the disease is difficult to determine without invasive biopsies. The condition is characterized by impairments in memory, cognition, language and mobility, and these impairments progress over time.

Post-mortem slices of brain tissue from AD victims show that amyloid-containing senile plaques are a prominent feature of selective areas of the AD and the Down Syndrome brain. Divry, P., *J. Neurol. Psych.*, 27: 643-657 (1927); Wisniewski, *et al.*, "Reexamination of the pathogenesis of the senile plaque," *In* Zimmerman, H.M. (ed.): *Progress in Neuropathology,* N.Y. (1973), Grune and Stratton, pp. 1-26. These plaques range in size from approximately 9 μm to 50 μm in diameter, when viewed by immunocytochemical methods designed to detect amyloid, and they vary in morphology and density. Majocha *et al.*, *Proc. Natl. Acad. Sci. USA*, 85: 6182-6186 (1988). Classical staining methods can detect senile plaques as large as 200 μm. Tomlinson, *et al.*, "Ageing and the dementias," *In*: Adams, J.H., *et al.*, (ed.), *Greenfield's Neuropathology*, Edition 4, J. Wiley and Sons, N.Y., pp. 951-1006. These plaques are most often found in the cerebral cortex, but they also occur in deeper grey matter, including the amygdaloid nucleus, the corpus striatum, and the diencephalon. Plaques have also been described in the cerebellum. Pro, *et al.*, *Neurology*, 30: 820-825 (1980). Senile plaques are composed of

extracellular amyloid, reactive cells, and degenerating neurites that contain Paired Helical Filaments, lysosomes, abnormal mitochondria and astrocytic processes. Wisniewski, *et al., supra* (1973). The mechanisms responsible for the excessive accumulation of amyloid, the major proteinaceous component of senile plaques, have been recently addressed at the protein chemistry, molecular biology and genetic level.

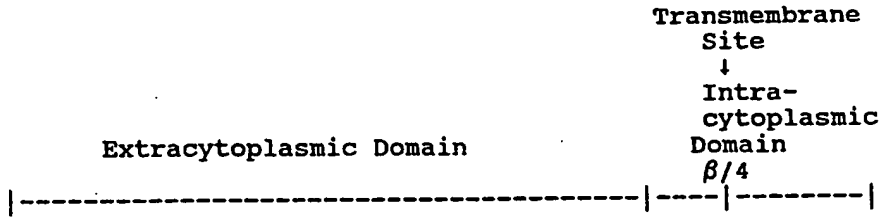
Specifically, amyloid is composed of fibrils of 4-8 nm in diameter that form the core of senile plaques. Mertz *et al., Acta Neuropathol.*, 60: 113-124 (1983). The amyloid is readily demonstrated by application of thioflavin S or Congo red to brain sections. In the latter case, polarized light causes amyloid to appear with a characteristic yellow-green color. The staining property reflects the presence of twisted beta-pleated sheet fibrils, as noted above. A detailed discussion of the biochemistry and histochemistry of amyloid can be found in Glenner, *N. Engl. J. Med.*, 302: 1333-1343 (1980).

Vascular amyloidosis, referred to as congophilic angiopathy, has been recognized since the early part of this century as a significant aspect of the microscopic pathology of Alzheimer's Disease. Vinters, *et al. Stroke*, 18: 311-324 (1987). Over 90% of Alzheimer cases have congophilic angiopathy. Glenner, *et al., Ann. Pathol.*, 1: 120-129 (1981). Similar to parenchymal amyloid deposits, vascular amyloid is demonstrated by characteristic thioflavin S and Congo red staining reactions. The parieto-occipital cortex is usually more affected than that in the frontal and temporal lobes. Tomlinson *et al., supra* (1984).

In vascular amyloidosis, the amyloid appears to infiltrate the micro-vasculature, and affected vessels often pass from the leptomeninges into the cortex. Small cerebral vessels with arterioles that appear as thickened

tubes are observed. The changes include the small pial and intracortical arterioles, the leptomeningeal vessels and the intracortical capillaries. Tomlinson *et al.*, *supra* (1984). Immunocytochemical and electron microscopic studies have indicated that the amyloid component of senile plaques are often observed in close proximity to affected microvessels. Allsop, *et al. Neurosci. Lett.*, 68: 252-256 (1986). However, the angiopathy may occur without senile plaques. Montjoy, *et al., J. Neurol. Sci.*, 57: 89-103 (1982).

The principle component of both cerebral (senile plaques) and vascular amyloid is the 4.2 kilodalton peptide, β-amyloid, which is also referred to as β/A4 and A4. Glenner *et al., Biochem. Biophys. Res. Commun.*, 120: 885 (1984). β/A4 is derived from a parent molecule, the amyloid precursor protein (APP). Kang *et al., Nature*, 325: 733-736 (1987). At least three major variants of APP are known, having 695, 751 and 770 amino acids, respectively. In all three variants, the site of the β/A4 peptide is in the same relative 3'-end location, as follows:



Kang *et al., supra* (1987) showed through cloning APP-695 that APP has a large extracellular domain, a transmembrane domain (which gives rise to the β/A4 peptide) and an intracytoplasmic domain (*See* Figure 9). The signal sequence, for transport through the endoplasmic reticulum membrane, is followed by a region rich in cysteine, which suggests that disulfide bridges may stabilize this portion of the structure. Within the next 100 residues are a stretch of 7 uninterrupted

threonine residues and a region containing 28 glutamic acid residues and 17 aspartic residues. Marotta, *et al. J. Mol. Neurosci.*, 3: 111-125 (1992) suggest that this domain could bind cations extensively and may have physiological significance. Sodium dodecyl sulfate ("SDS") may be bound to a lesser extent than usual due to this domain. The region from residue 290 to 597, at which point the β/A4 site begins, contains two potential N-glycosylation sites at positions 467-469 and 496-498. The β/A4 peptide (residues 596-638 or 639) is either 42 or 43 amino acids in length and partly includes the putative transmembrane domain (amino acids 625-648). The C-terminal region of the APP is relatively small, consisting of 57 residues.

Following the transmembrane region, lysine residues are present (residues 649-651) which, according to Kang *et al. supra* (1987), could interact with phospholipid head groups in the membrane. This feature has been described for the junction between membrane and cytoplasmic domains of cell-surface receptors. One site (amino acids 684-686) is a potential glycosylation sequence.

Gandy *et al.* report that during in vitro studies of synthetic peptides corresponding to the cytoplasmic domain, it was observed that protein kinase C rapidly catalyzed the phosphorylation of a peptide corresponding to amino acid residues 645-661 on ser-655. Gandy *et al.*, *Proc. Natl. Acad. Sci. USA*, 85(16): 5218-5221 (1988), suggesting that this site may be an important control region for amyloid metabolism and its interaction with other intracellular regulatory elements.

Recent research has also focused on the biological activity of β/A4. Specifically, it has been noted that this peptide and its fragments are trophic, toxic and or amnestic at various concentrations. Also, β/A4 forms insoluble aggregates (self-aggregates) under various

conditions, and the neurotoxicity of β/A4 is related to the aggregation process. Kirshner, *et al.*, *Proc. Natl. Acad. Sci. USA*, 84: 6953-6957 (1987) and Maggio, *Annu. Rev. Neurosci.*, 11: 13-28 (1988). Maggio *et al.*, also studied the aggregation properties of radioiodinated synthetic β/A4 peptides *in vitro*. *Proc. Natl. Acad. Sci. USA*, 89: 5462-5466 (June 1992).

Pike *et al.*, *J. Neurosci.*, 13(4): 1676-1687 (1993) tested the aggregation properties of an overlapping series of synthetic ß-amyloid peptides and compared them with their neurotoxic properties *in vitro*. They discovered that few peptides assembled into aggregates immediately after solubilization but that over time peptides containing the highly hydrophobic ß29-35 region formed stable aggregations. In short-term cultures, neurotoxicity was associated with those peptides demonstrating significant aggregations.

Thus far, diagnosis of AD has been achieved mostly through clinical criteria evaluation, brain biopsies and post mortem tissue studies. However, recent work has focused on immunoassay methods for detecting markers of AD in body fluids such as spinal fluid and also in *in situ* hybridization studies using nucleic acid probes. World Patent No. 92/17152 by Potter; Warner, M., *Anal. Chem.*, 59: 1203A (1987); U.S. Patent No. 4,666,829 by Glenner *et al.* In U.S. application no. 105,751, the contents of which is hereby incorporated by reference, Marotta *et al.* describe anti-β/A4 antibodies for purposes of *in vitro* and *in vivo* diagnostic methods.

Glenner *et al.*, *supra*, teach the use of the ß/A4 peptide, or fragments thereof, for the production of antibodies which recognize the antigenic determinants of the polypeptide or homologues thereof. Glenner *et al.* further teach the use of the disclosed polypeptide for the production of nucleic acid probes which hybridize with the gene encoding the polypeptide. One such

polypeptide has the following amino acid sequence (SEQ ID NO:1): H_2N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Gln-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-COOH. The diagnostic methods taught in this patent are characterized as non-invasive.

U.S. Patent Nos. 5,039,511 and 4,933,156 by Quay *et al.* describe the *in vitro* and *in vivo* use of iodinated imaging compounds derived from bisdiazobenzidine compounds to detect the presence and location of amyloid deposits in an organ or area of a patient.

Although ß/A4 has been considered for use in *in vitro* diagnostic methods, this polypeptide has never been described in connection with *in vivo* diagnostic imaging methods. Therefore, a need exists for a diagnostic *in vivo* imaging method that exploits the self-aggregation properties of amyloid proteins such as ß/A4.

Summary of the Invention

One object of the present invention is to provide an amyloid binding composition for *in vivo* imaging of amyloid deposits comprising a labeled amyloid protein which binds to amyloid deposits *in vivo* and a pharmaceutically acceptable carrier.

Another object of the present invention is to provide an *in vivo* method for detecting amyloid deposits in a subject comprising the steps of administering to a subject a detectable quantity of an amyloid binding composition comprising a labeled amyloid binding protein and a pharmaceutically acceptable carrier and detecting the binding of the labeled protein to the amyloid deposit.

Another object of the present invention is to provide a method of diagnosing an amyloidosis-associated disease, such as Alzheimer's Disease and Down Syndrome, by applying the above method to the detection of amyloid deposits in subjects suspected of having an amyloidosis-associated disease.

The amyloid binding protein of the present invention includes all variants of the amyloid protein which bind to amyloid deposits *in vivo*.

Brief Description of the Drawings

Figure 1 shows a chart setting forth chemically documented amyloidosis with protein types.

Figure 2 depicts a PAGE-SDS gel of the A4-O synthetic amyloid peptide. The amyloid polypeptide of 28 residues, corresponding to the previously reported sequence of Masters *et al.*, *Proc. Natl. Acad. Sci. USA* 82: 4245-4249 (1985) was synthesized on a Biosearch SAM2 synthesizer using the general procedure of Merrifield, *J. Am. Chem. Soc.*, 85: 2149-2154 (1963). Purification was achieved with a 3 X 65 cm column of Sephadex G50 (10-40 μ). The peptide (10 μg) was suspended in sample buffer containing 2% SDS (Brown, *et al.*, *J. Neurochem.*, 40: 299-308 (1983))

and 9.5 M urea. Electrophoresis was carried out n a uniform 10% gel containing 0.1% SDS. (A) Molecular weight markers: phosphorylase B (94 kd), bovine serum albumin (68 kd), ovalbumin (43 kd), carbonic anhydrase (29 kd), trypsin inhibitor (22 kd), lysozyme (14.5 kd). (B) The synthetic amyloid peptide ran as a sharp band at the front of the gel and as an aggregated form of higher molecular weight.

Figure 3 depicts gel electrophoresis of the synthetic peptides A4-O and P2 (APP amino acids 413-429). (Panel a): Twenty ug of A4-O (lane 1) and P2 (APP amino acids 413-429) (lane 2) were analyzed by 18% SDS-PAGE (acrylamide : bisacrylamide = 30 : 0.8). (Panel b): Analysis of A4-O and P2 (APP amino acids 413-429) on 11% SDS/urea-PAGE (acrylamide : bisacrylamide = 20 : 1). Lanes 1-3 containing A4-O (10 ug) were incubated with 2% SDS and 5% 2-ME at 95°C for 5 minutes (Lane 1), 30 min (Lane 2) and 60 minutes (Lane 3). Lane 4 contained P2 (10ug). Gels were stained with Coomassie Brilliant blue R-250. Molecular weights are shown on the right (Kd).

Figure 4 shows slot blots of immunostained A4-O after addition of itself or a second A4 homologue. This assay depicts the increase staining intensity after A4 homologues are added to one another. This reflects the ability of homologues to self-aggregate and thus increase the staining intensity. In each case, the slot contained 1 ug of A4 peptide. To each, 2.5, or 10.0 ug of exogenous peptide was added, as indicated. The blots were then immunostained (see descriptions of Figures 5, 6 and 7).

Figure 5 shows immunoblots with and without exogenous A4-O peptide. Density values of the immunoreaction products of A4-O with and without exogenous peptides after reaction with 10H3. The values of the bars correspond to the density of blots shown in Figure 4. The description of Figure 4 indicates the condition of

each blot with regard to the exogenous peptide that was added to the blotted peptide prior to addition of 10H3. The height of the bars above the black bar (no peptide addition) is a measure of the extent to which the exogenous peptide bound to the attached peptide on the filter paper and increased the density of immunostaining of the complex.

At the three indicated concentrations (2.5, 5.0 and 10.0 ug/ml) A4-O was added to the A4-O that was already present at a concentration of 1 ug. The blot was then immunostained to develop the colored reaction product. The data were derived from scanning Figure 4.

Figure 6 shows immunoblots with and without exogenous A4-H peptide. At the three indicated concentrations (2.5, 5.0 and 10.0 ug/ml) A4-H as added to the A4-O that was already present at a concentration of 1 ug. The blot was then immunostained to develop the colored reaction product. The data were derived from scanning Figure 4. See description of Figure 5 for more details.

Figure 7 shows immunoblots with and without exogenous Op1 peptide. At the three indicated concentrations (2.5, 5.0 and 10.0 ug/ml) Op1 as added to the A4-O that was already present at a concentration of 1 ug. The blot was then immunostained to develop the colored reaction product. The data were derived from scanning Figure 4. See description of Figure 5 for more details.

Figure 8 shows the reactivity of 10H3 towards A4-O (upper panel).

Figure 9 (SEQ ID NOS:11 and 12) is the nucleotide sequence and predicted amino acid sequence of cDNA encoding the precursor protein (APP) of the β/A4 with the β/A4 region boxed, as set forth in Kang *et al.*, *supra*, (1987).

Detailed Description of the Preferred Embodiments

Applicants have discovered that an amyloid binding composition comprising a labeled amyloid protein may be used *in vivo* for detecting the presence and location of amyloid deposits. The amyloid binding composition of the present invention comprises a labeled amyloid protein and a pharmaceutically acceptable carrier. This protein is any natural or synthetic protein which binds to amyloid deposits *in vivo*. In one embodiment, the protein is the β-amyloid polypeptide (ß/A4 peptide), which in its longest form has 42 to 43 amino acid residues, as shown in Figure 9. *See* Masters, *et al.*, *Proc. Nat. Acad. Sci., USA.*, 82: 4245-4249 (1985).

As noted above, ß/A4 is derived from a larger amyloid precursor protein having from 695 to 770 amino acids. *See* Kang *et al.*, *Nature*, 325: 733 (1987). The term "amyloid deposit" includes amorphous, eosinophilic materials that are associated with amyloidosis, a disease complex including over 20 different clinically defined syndromes, as discussed above. Chemically, amyloid deposits are proteinaceous, and their chemical compositions are unique for each of the clinical syndromes with which they are associated, as set forth in Figure 1. Preferably, the amyloid deposit of the present invention is that found in the brain of Alzheimer's Disease patients. As noted above, such amyloid deposits are found in senile plaques in selected areas of the AD brain and are composed of fibrils of 4-8 nm diameter. These plaques are detected by application of thioflavin S or Congo red to brain sections and in the latter case, appear yellow-green under polarized light. They have twisted beta-pleated sheet fibrils and are further characterized by Glenner, *N. Eng. J. Med.*, 302: 1333-1343 (1980). In another embodiment, the amyloid deposit of the present invention is that which is associated with vascular amyloidosis, as

described in Vinters, *Stroke*, 18: 311-324 (1987). Vascular amyloid deposits infiltrate the cerebral microvasculature. Similar to amyloid deposits found in senile plaques in the parenchyma of the AD brain, vascular amyloid deposits have characteristic thioflavin S and Congo red staining reactions. Montjoy *et al.*, *J. Neurol. Sci.*, 57: 89-103 (1988).

The term "amyloidosis-associated disease" includes any disease characterized by local or systemic amyloid deposits. (*See* Figure 1) Preferably, the amyloidosis-associated disease of the present invention is Alzheimer's Disease or Down Syndrome.

In addition to amyloid protein purified from natural sources such as cerebrovascular tissue, as described hereinafter, amyloid protein of the present invention includes recombinant and synthetic amyloid protein and variants of the naturally occurring, recombinant and synthetic protein. In a preferred embodiment, the amyloid protein of the invention comprises the β-amyloid polypeptide and variants thereof. The category of "variants" includes, for example, a fragment of the β-amyloid polypeptide or any homologous amino acid sequence or amino acid addition, wherein the resulting polypeptide has the same or similar function as the natural occurring polypeptide in that it binds to amyloid deposits *in vivo*. In one embodiment, the amyloid protein of the present invention is comprised of the β-amyloid polypeptide or variant thereof and amino acids from the APP protein which are from regions of the APP protein which are either adjacent or non-adjacent to the β-amyloid polypeptide. For example, in one embodiment, the amyloid protein of the present invention comprises:

(A) The β/A4 peptide alone (SEQ ID NO:2):

Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-

Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr; or

(B) The β/A4 peptide plus the amino acids of the transmembrane domain of the APP (SEQ ID NO:3):

Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr-Val-Ile-Val-Ile-Thr-Leu-Val-Met-Leu; or

(C) The β/A4 peptide plus the remaining C-terminal amino acids of the entire APP (SEQ ID NO:4):

Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr-Val-Ile-Val-Ile-Thr-Leu-Val-Met-Leu-Lys-Lys-Lys-Gln-Tyr-Thr-Ser-Ile-His-His-Gly-Val-Val-Glu-Val-Asp-Ala-Ala-Val-Thr-Pro-Glu-Glu-Arg-His-Leu-Ser-Lys-Met-Gln-Gln-Asn-Gly-Tyr-Glu-Asn-Pro-Thr-Tyr-Lys-Phe-Phe-Glu-Gln-Met-Gln-Asn; or

(D) The β/A4 peptide with the preceeding 10 amino acids of the APP (SEQ ID NO:5):

Lys-Thr-Glu-Glu-Ile-Ser-Glu-Val-Lys-Met-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr; or

(E) The β/A4 peptide with any other APP amino acids attached to it that are not normally ajacent (SEQ ID NO:6):

X-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr-Y,

wherein X and Y are one or more APP amino acids which are not ajacent to β/A4 in the nature; and

(F) any fragment of (A)-(E), wherein said fragment is large enough to bind amyloid deposit *in vivo*.

The term "fragment" includes a linear amino acid subsequence of the ß-amyloid polypeptide, wherein such fragment binds amyloid deposits *in vivo*. A variant which contains an amino acid sequence variation or substitution is a homologous sequence. "Homology" between two sequences connotes a likeness short of identity indicative of a derivation of the first sequence from the second. For example, a polypeptide is "homologous" to ß-amyloid polypeptide if it contains an amino acid sequence similar enough to the natural sequence so as to confer the same or similar amyloid binding property as the natural ß-amyloid polypeptide. Such a sequence may be only a few amino acids long and may be a single linear sequence or one or more linear sequences which confer binding activity to the polypeptide when amino acids from separated portions of a linear sequence are spatially juxtaposed after protein folding. The variants encompassed by this invention can be ascertained, for example, by the *in vitro* quantitative assays describe below in Examples 3-7. That is, applicants have conducted a series of studies involving the addition of increasing concentrations of ß-amyloid polypeptide variants to a solid support containing a specific peptide called A4-0. The increase in density of immunostain using an anti-A4-0 monoclonal antibody, 10H3, described in U.S. Patent application no. 105,751 by Marotta, *et al.*, incorporated by reference above, was measured. Based upon this work, it was possible to determine which peptides were suitable for use in the *in vivo* methods, according to the invention. Other poly- and/or monoclonal antibodies suitable for this assay can be produced by methods well known in the art. *See* Kennett *et al.*, *Monoclonal Antibodies- Hybridomas: A New Dimension in Biological Analysis*, Plenum Press (1980)

Protein which qualifies as "amyloid protein" according to the above criteria can be produced by

methods known and emerging in the art, including conventional reverse genetic techniques, i.e., by designing a genetic sequence based upon an amino acid sequence or by conventional genetic splicing techniques. For example, ß-amyloid polypeptide variants can be produced by techniques which involve site-directed mutagenesis or oligonucleotide-directed mutagenesis. See, for example, "Mutagenesis of Cloned DNA," in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY 8.0.3 *et seq.* (Ausubel, *et al.* eds. 1989) ("Ausubel").

Other amyloid protein variants within the present invention are molecules that correspond to a portion of the ß-amyloid polypeptide, but are not coincident with the natural molecule, and display the binding activity of the natural molecule when presented alone or, alternatively, when linked to a carrier or biologically active signal sequence that permits proteins to pass through membranes. *See* von Heijne, G., *J. Mol. Biol.*, 184: 99-105 (1985). An amyloid protein variant of this type could represent an actual fragment, as discussed above, or could be a polypeptide synthesized *de novo* or recombinantly.

To be used in recombinant expression of amyloid protein or amyloid protein variant, a polynucleotide molecule encoding such a molecule would preferably comprise a nucleotide sequence, corresponding to the desired amino acid sequence, that is optimized for the host of choice in terms of codon usage, initiation of translation, and expression of commercially useful amounts of, for instance, ß-amyloid polypeptide or ß-amyloid polypeptide variant. Also, the vector selected for transforming the chosen host organism with such a polynucleotide molecule should allow for efficient maintenance and transcription of the sequence encoding the polypeptide. The encoding polynucleotide molecule may code for a chimeric protein; that is, it can have a

nucleotide sequence encoding a biologically active part of the ß-amyloid molecule operably linked to a coding sequence for a non-ß-amyloid moiety, such as a signal peptide for the host cell.

For instance, in order to isolate a DNA segment which encodes a ß-amyloid molecule, total DNA from cerebrovascular tissue can be prepared according to published methods. See, for example, Maniatis, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL (Cold Spring Harbor Laboratories, NY 1982); Baess, *Acta Pathol. Microbiol. Scand.* (Sect. B), 82: 78084 (1974). The DNA thus obtained can be partially digested with a restriction enzyme to provide an assortment of genomic fragments. An enzyme with a tetranucleotide recognition site, such as *Sau*3A (*Mbo*I), is suitable for this purpose. The fragments from such a partial digestion then can be size-fractionated, for example, by sucrose gradient centrifugation (see Maniatis, *supra*) or by pulsed field gel electrophoresis (See Anad, *Trends in Genetics*, November 1986, at pages 278-83), to provide fragments of a length commensurate with that of DNA encoding the ß-amyloid molecule. Molecular cloning of amyloid cDNA derived drom mRNA of the Alzheimer brain and the expression thereof is described in detail in Zain *et al.*, *Proc. Natl. Acad. Sci. USA.*, 85: 929-933 (1988) and Marotta *et al.*, *Proc. Natl. Acad. Sci. USA.*, 86: 337-341 (1989), respectively, both of which are herein incorporated by reference.

According to well-known methods described, for example, in Ausubel at 5.0.1 et seq., the selected fragments can be cloned into a suitable cloning vector. A DNA sequence thus obtained could be inserted, for example, at the *Bam*H1 site of the pUC18 cloning vector which is transfected into appropriate host cells such as *E. coli* or a mammalian cell. A variety of screening

mechanisms known in the art of the invention can then be used to identify clones containing the ß-amyloid gene.

In another embodiment of the invention, amyloid protein of the present invention is purified from tissue samples. For example, brains from Alzheimer's Disease post-mortum patients are histologically sectioned and stained with Congo Red dye. Upon visualization with a polarizing microscope, amyloid deposits can be identified by their green color. Brains exhibiting extensive cerebrovascular amyloidosis are used as source for purified amyloid protein. After removal of contaminants from the amyloid containing vessels of the meninges, the meningeal tissues are homogenized and centrifuged to yield a brownish layer rich in amyloid fibrils. This layer is then digested with collagenase, solubilized in 6M guanidine HCl, pH 8.0 and centrifuged. The supernatant containing the solubilized protein is desalted by dialysis and gel exclusion column chromatography and high performance liquid chromatography is used to purify the polypeptide. The amino acids for the purified protein (e.g., ß-amyloid polypeptide) are then sequentially cleaved in an automated amino acid sequencer, such as a Beckman 890 C spinning cup sequencer, and analyzed by high performance liquid chromatography in order to determine the amino acid sequence of the amyloid protein. *See* Glenner & Wong, *Biochem. Biophys. Chem. Res. Commun.*, 120: 885 (1984).

In another embodiment, amyloid protein and variants thereof can be produced in accordance with published methods. For instance, Kirschner *et al.*, *Proc. Natl. Acad. Sci. USA*, 84: 6953-57 (1987) used an ABI Synthesizer model 380 B (Applied Biosystems, Foster City, CA) to synthesize synthetic ß-amyloid peptides consisting of residues 1-28 and homologues thereof. General methods for peptide synthesis can be found in Clark Lewis *et al.*, *Science*, 231: 134 (1986). *See also*, Hilbich *et al.*, *J.*

Mol. Biol., 218: 149-163 (1991); Majocha *et al.*, *Proc. Natl. Acad. Sci. USA*, 85: 6182-6186 (1988); and U.S. Patent application No. 105,751 by Marotta *et al.*

The term "*in vivo* imaging" refers to any method that permits the detection of a labeled amyloid protein which binds to amyloid deposits located in a subject's body. A "subject" is a mammal, preferably a human. Often, particularly when the composition and method of the invention is directed to the diagnosis of Alzheimer's Disease or Down Syndrome, the subject will manifest clinical symptoms of the suspected amyloidosis. These clinical symptoms are well-known to the practitioner of this invention and include loss of memory, and other impairments described above.

The amyloid binding composition of the present invention must be of a "detectable quantity." A detectable quantity is that which is sufficient to enable detection of the site of amyloid deposit location when compared to a background signal. The dosage of the amyloid binding composition will vary depending upon such considerations as age, condition, sex, extent of disease in the patient, counterindications, and other variables, to be adjusted by the individual physician. Dosage can vary from 0.01 mg/kg to 2,0000 mg/kg, preferably 0.1 mg/kg to 1,000 mg/kg.

In accordance with this invention, the amyloid protein may be labeled by any of several techniques known to the art. *See, e.g.*, Wagner *et al.*, *J. Nucl. Med.*, 20: 428 (1979); Sundberg *et al.*, *J. Med. Chem.*, 17: 1340 (1974) and Saha *et al.*, *J. Nucl. Med.*, 6: 542 (1976).

The label is chosen based upon the type of detection instrument employed. For instance, a chosen radionucleotide must have a type of decay which is detectable for a given type of instrument. Another

consideration relates to the half-life of the isotope. The half-life should be long enough so that it is still detectable at the time of maximum uptake by the target, but short enough so that the host does not sustain deleterious radiation. Preferably, the chosen label will lack a particulate emission, but will produce a large number of photons in a 140-200 keV range, which may be readily detected by, for instance, conventional gamma camera. Suitable radioisotopes for purposes of this invention include, gamma-emitters, position-emitters, x-ray emitters and fluorescence-emitters. These radioisotopes include Iodine-131, Iodine-123, Iodine-126, Iodine-133, Bromine- 77, Indium-111, Indium-113m, Gallium-67, Gallium-68, Ruthenium-95, Rutheium-97, Ruthenium-103, Ruthenium-105, Mercury-107, Mercury-203, Rhenium-99m, Rhenium-105, Rhenium 101, Tellurium-121m, Tellurium-122m, Tellurium-125m, Thulium-165, Thulium-167, Thulium-168, Technetium-99m and Fluorine-18. The preferred radiolabel is Technetium-99m. Suitable paramagnetic isotopes for use in Magnetic Resonance Imaging (MRI), according to this invention, include ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr, and ^{56}Fe.

Administration to the subject may be accomplished intraventricularly, intravenously, intraarterially, via the spinal fluid or the like. Administration may also be intradermal or intracavitary, depending upon the body site under examination. After a sufficient time has lapsed for the labeled amyloid protein to bind with amyloid deposits, for example 30 minutes to 48 hours, the area of the subject under diagnosis is examined by routine imaging techniques such as MRI, SPECT and planar scintillation imaging. The exact protocol will necessarily vary depending upon factors specific to the patient, as noted above, and depending upon the body site under examination, method of administration and type of label used; the determination of specific procedures

would be routine to the skilled artisan. The distribution of the bound radioactive isotope and its decrease with time is then monitored and recorded. By comparing the results with data obtained from studies of clinically normal individuals, the presence and location of amyloid deposits can be determined.

Thus, in one embodiment, the methods of the present invention is used to diagnoses an amyloidosis-associated disease. Where the site of examination is the brain, the *in vivo* detection of amyloid deposits according to the methods of the present invention signifies a diagnosis of Alzheimer's Disease. The detection of amyloid deposits in the brain of patients manifesting clinical symptoms of Down Syndrome, signifies a diagnosis of Down Syndrome. In that regard, applicants note that the gene for APP, located on chromosome 21, is over-represented in Down Syndrome individuals (Serra *et al.*, *Amer. J. Med. Gen. Supp.*, 7: 11-19 (1990). Accumulations of amyloid occur in young Down Syndrome patients, with nearly 90% of Down Syndrome subjects aged less than 30 years showing amyloid accumulation (*Hyman, Prog. Clin. Biol. Res.* 379: 123-142 (1992)). The Down Syndrome patient displays amyloid accumulations early in life, often by late teenage years. As adults, nearly 100% will develop Alzheimer Disease (*Cork, Amer. J. Med. Gen. Supp.*, 7: 282-539 (1990)). The neuropathology of Down Syndrome is essentially identical to that of Alzheimer Disease and includes β/A4 amyloid deposits in senile plaques. The Alzheimer - like lesions represent a major neuropathologic trait of the brain of the Down Syndrome patient (Serra *et al.*, *Supra* (1990)).

The amyloid-binding compositions of the present invention are advantageously administered in the form of injectable compositions. A typical composition for such purpose comprises a pharmaceutically acceptable carrier. For instance, the composition may contain about 10 mg of

human serum albumin and from ab ut 20 to 200 micrograms of the labeled amyloid protein per milliliter of phosphate buffer containing NaCl. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described in REMINGTON'S PHARMACEUTICAL SCIENCES, 15th Ed. Easton: Mack Publishing Co. pp 1405-1412 and 1461-1487 (1975) and THE NATIONAL FORMULARY XIV., 14th Ed. Washington: American Pharmaceutical Association (1975), the contents of which are hereby incorporated by reference. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyloleate. Aqueous carriers include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, etc. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobials, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components of the binding composition are adjusted according to routine skills in the art. *See* GOODMAN AND GILMAN'S THE PHARMACOLOGICAL BASIS FOR THERAPEUTICS (7th ed.). The skilled artisan would also readily appreciate that a suitable excipient or carrier would need to prevent aggregation of the binding composition prior to contacting the target amyloid deposit *in vivo*.

Particularly preferred amyloid binding compositions of the present invention are those that, in addition to binding to amyloid deposits in *in vivo*, are also non-toxic at appropriate dosage levels, have a satisfactory duration of effect, and display an adequate ability to cross the blood-brain barrier. In this regard, United States Patent No. 4,540,564 discloses an approach for enhancing blood-brain barrier-penetrating ability by attaching a centrally acting drug species to a reduced,

biooxidizable, lipoidal form of dihydropyridine pyridinium salt redox carrier. Thus, in one embodiment, the composition of the present invention includes such a blood-brain barrier crossing enhancer carrier.

In vivo animal testing provides yet a further basis for determining dosage ranges, efficacy of transfer through the blood barrier and binding ability. Particularly preferred for this purpose is the "senile animal" model for cerebral amyloidosis -- animals such as aged dogs or monkeys, which are known to develop variable numbers of Alzheimer-type cerebral senile plaques, *see* Wisniewski, *et al.*, *J. Neuropathol. & Exp. Neurol.*, 32: 566 (1973), Selkoe, *et al.*, *Science*, 235: 873 (1987) are tested for binding and detection efficacy. This *in vivo* assay requires control-biopsy monitoring to confirm and quantify the presence of amyloid deposits.

Also, cellular models of amyloidosis have been prepared that overproduce β-amyloid polypeptide in animals for purposes of testing the efficacy of the amyloid binding compositions and methods of the present invention. *See* Marotta, *et al. Proc. Natl. Acad. Sci. USA,* 86: 337-341 (1989). Such cell models have been adapted to a behavior paradigm. *See* Tate-Ostroff, *Proc. Natl. Acad. Sci. USA* 89: 7090-7094, (1992). That is, because AD patients suffer circadian rhythm dysfunction, this behavioral deficit was modeled in rats by a cell grafting techniques. PC12 cells transfected with the β-amyloid polypeptide C-terminal region of the APP were implanted into the suprachiasmatic nuclei ("SCN") of rats; the SCN is a primary circadian oscillator in mammals. Animals receiving amyloidotic cell grafts, but not animals receiving control cell grafts, exhibited disrupted activity rhythms, although temperature rhythms were unaffected. The specificity of the disruption was similar to circadian dysfunction seen in AD patients. The data supported an association between a defined

behavioral disruption and amyloid overexpression either directly or through the release of cellular factors as a consequence of amyloid overproduction.

Other suitable animal models for use in testing the compositions and methods of the present invention are produced transgenically. For instance, Quon *et al.*, *Nature*, 352: 239-241 (1991) used rat neural-specific enolase promoter inhibitor domain to prepare transgenic mice. *See also*, Wirak *et al.*, *Science*, 253: 323-325 (1991). Still other models have been produced by Intracranial administration of the β/A4 peptide directly to animals (Tate *et al.*, *Bull. Clin. Neurosci.*, 56: 131-139 (1991).

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following examples are merely illustrative and not limitative of the remainder of the disclosure in any way whatsoever.

EXAMPLES

As noted above, A4 is intended to be the same as β/A4, throughout the examples. The peptides used in the following Examples have the following structures:

A4-O (peptides 1-28), SEQ ID NO:7:

N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Ser-Ala-COOH

The A4-O(1-28) polypeptide that was reported in Masters, *et al. Proc. Nat'l. Acad. Sci. U.S.A.*, *82*: 4245-4249 (1985) is the first 28 amino acids of the 4.2 Kd peptide derived from senile plaque cores of an AD brain. Masters, *et al.* have also shown that the naturally occurring peptide aggregates even in denaturing gels. The A4-O(1-28) sequence of this invention was synthesized by Biosearch in San Rafael, CA. The underlined amino acids differ from A4-P(1-28), as shown below.

A4-H (peptides 1-28):

The A4-H peptide is the same as A4-O(1-28) except that it was synthesized by the Harvard Microchemistry Laboratory.

A4-P (peptides 1-28), SEQ ID NO:8:

N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Gln-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-COOH

This sequence was reported by Glenner and Wong, *supra*, (1984) and derived from vascular amyloid of the AD brain and from a Down Syndrome brain. Three of 28 amino acids are different from the A4-O/A4-H peptides(underlined).

A4-B (peptides 1-28), SEQ ID NO:9:

N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-COOH

This sequence was obtained from Bachem and is the 28 amino acid structure that is commonly determined from

molecular cloning studies (Kang, *et al.*, *Nature (London) 325:* 733-736 (1987)). Unlike the Glenner and Wong, *supra*, sequence (A4-P(1-28)), it has Glu, not Gln, at position 11. And, unlike A4-O/A4-H, it has Asn-Lys and not Ser-Ala at the C-terminus.

Op1 (peptides 1-10), SEQ ID NO:10:

N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-COOH

A4(1-10) consists of the first 10 amino acids of the amyloid peptide derived from any source and is described in U.S. Patent No. 4,666,829 by Glenner *et al.* Thus far, this sequence appears conserved in all reports on amyloid that is derived from non-Familial AD cases. The A4-(1-10) antigen used in the present studies was synthesized by the Harvard Microchemistry Laboratory.

Summary of sequence variations: dashed line indicates sequence conservation among the peptides shown.

```
A4-O (1-28): N----------Glu---------------Ser-Ala-| (Masters)
A4-H (1-28): N----------Glu---------------Ser-Ala-| (Masters)
A4-P (1-28): N----------Gln---------------Asn-Lys-| (Glenner)
A4-B (1-28): N----------Glu---------------Asn-Lys-| (Kang)
Op1        : N---------|
```

Example 1. Self-aggregation of the A4-O amyloid peptide in SDS/urea acrylamide gels

The synthetic ß-amyloid 28-mer polypeptide, A4-O (Masters, *et al.*, *supra*.) was analyzed by polyacrylamide gel electrophoresis (PAGE) procedures (Brown, *et al.*, *J. Neurochem.*, *40*: 299-308 (1983)) and was noted to have unusual aggregation properties. The peptide was dissolved in a PAGE sample buffer containing SDS and urea and was electrophoresed on a 10% gel containing SDS (See description of Figure 2). After staining with Coomassie blue, the peptide appeared as a broad band at approximately 23-25 kd and a narrow band that migrated at the get front during electrophoresis (See Figure 2). The higher molecular weight species appeared to be an aggregate since it was eliminated by adding urea to the separating get and, subsequently, a 3-4 kd band was obtained (not shown). Polyclonal antiserum to the 28-mer was prepared and applied to nitrocellulose blots of an overloaded gel. The latter contained a series of aggregated peptides of various apparent molecular weights, all of which reacted with the antiserum. Thus, the synthetic 28-mer had aggregational properties not unlike the naturally occurring A4-O amyloid protein of 4 kd (Masters, *et al.*, *supra*).

Applicants' studies demonstrating the aggregation properties of the A4-O peptide were previously reported (Salim, *et al.*, "Molecular Cloning of Amyloid cDNA from Alzheimer Brain Messenger RNA" in *Familial Alzheimer's Disease*, J.P. Blass *et al.* eds., Marcel Dekker, NY pp 153-165 (1988).

Based upon the results shown in Figure 2, applicants concluded that even in the presence of strong denaturing agents and after electrophoresis, A4-O strongly bound to itself.

Example 2: Self-aggregation of A4-O peptide on highly cross-linked SDS/urea acrylamide gels

Applicants obtained confirmatory data using the highly cross-linked acrylamide gel system described by Honda and Marotta, *Neurochem. Res.*, 17: 367-374 (1992).

When analyzed by this gel system containing SDS and urea, the synthetic peptide A4-O migrated as a broad series of bands below an apparent molecular weight of 15kDa (data not shown). However, when 6M urea was added to the PAGE system the peptide appeared as a sharp single band of 15kDa (Figure 3) and smaller size bands were not observed even after silver staining (data not shown). By contrast, peptide P2(413-429), used as a control and corresponding to an extracytoplasmic region of the β/A4 precursor protein, migrated with the bromphenol blue dye front on both SDS-PAGE and SDS/urea-PAGE systems (Figure 3, lane 4). Since the theoretical molecular weight of the 28 amino acid peptide A4-O is 3,178 Da the results indicate that the band of 15kDa is an aggregate. Migration of A4-O peptide bands on both gel systems was not affected by 2-ME nor by pre-treatment with 80% formic acid (data not shown).

The 15kDa was visible after peptide A4-O were treated for 5 minutes at 95°C prior to electrophoresis (Figure 3, lane 1). When boiling time was increased to 30 minutes or 60 minutes the aggregate partly dissociated to a smaller size (Figure 3, lanes 2 and 3). This dissociation was not dependent on the presence of SDS and 2-ME in the sample buffer but rather on the time of heat denaturation. These data were previously reported (Honda and Marotta, *supra*).

Applicants concluded that Figure 3 confirms that even in the presence of strong denaturing agents and heat treatment after electrophoresis, A4-O strongly bound to itself.

Example 3: Self-aggregation of A4 peptides on immunoblots

Due to the desirability of obtaining a quantitative assay for the selection of ß-amyloid polypeptides for the composition and methods of the present invention, applicants elected to use quantitative slot blots to test aggregation of peptides rather than tissue slices. The general immunoblotting procedure utilizing A4 peptides attached to a solid support and detectable by applied anti-amyloid antibodies was reported earlier (Majocha, *et al.*, *supra*, (1988). In all cases, the monoclonal antibody used to detect A4 aggregates was 10H3 (2 ug/ml).

One microgram of each of the indicated peptides were added overnight at room temperature to Millipore P filter paper to which A4-O was attached. The peptides were dissolved in ICC buffer: 2% BSA, 0.3M NaCl, 20mM Tris, 0.01% Triton. The blots were immunoprocessed (Majocha, *et al. supra.*) and then optically scanned for density; the areas under the curves were integrated by means of an LKB Laser Densitometer.

Peptides A4-O, A4-H and Op1 were applied to filters to which was bound peptide A4-O, the antigen used to prepare mab 10H3. The experiment was designed to test the competence of each of the applied peptides to bind to the bound peptide. While A4-O and A4-H have the same primary structure, it has been noted that peptides with identical sequences that are obtained from different sources may have non-identical properties. (See Figure 4).

The density of staining (the optical density of the immunoreaction product) is quantitated in Figures 5, 6 and 7. The OD is a measure of the extent of the aggregation since it will be related to the antibody concentration and thus the color reaction.

The density values shown in Figure 5 were obtained by densitometric scanning of the reaction product on

blots from which the control value (no primary antib dy) was subtracted. A further control was one in which the mab 10H3 was added to blots containing Op1 in the absence of added exogenous peptide. This control value represents the antibody-antigen reaction without interference from added peptides.

Based upon the results presented in Figure 5, applicants concluded that A4-O bound to itself with at an optimal concentration of 5.0 ug/ml.

Example 4: Self-aggregation of A4-H peptides on immunoblots

The experiment of Example 3 was repeated except that the exogenous peptide was A4-H. The data are shown in Figure 6 and based upon these results, applicants concluded that A4-H bound to A4-O at an optimal concentration of 2.5 ug/ml.

Example 5: Self-aggregation of Op1 peptides on immunoblots

The experiment of Example 3 was repeated except that the exogenous peptide was Op1. The data are shown in Figure 7 and based upon these results, applicants concluded that Op1 bound to A4-O at an optimal concentration of 2.5 ug/ml.

Based upon the results presented in Figures 5, 6 and 7, applicants concluded that three peptides bound the filter-bound A4-O peptide and increased the extent to which 10H3 reacted. The reaction is concentration-dependent. The three peptides, A4-O, A4-H and Op1, aggregated to the attached A4-O. The Op1 10-mer reacted nearly as well or better, at 2.5 ug, as the larger 28-mers.

Example 6: Specificity of 10H3 for both A4-O and Op1

The results shown in Figure 7 indicate that a small peptide, a ten-mer, was able to bind at least as well as 1-28-mers to an A4 substrate.

Thus, this assay, which measures the optical density of the reaction product between the added 10H3 mab and the Op1 peptide on the solid surface, reflected the presence of the exogenous peptide, as applicants previously demonstrated for the reaction between 10H3 and A4-O.

With respect to Op1, additional studies were carried out to confirm the reactivity of 10H3. On separate solid supports (Millipore P paper) either the A4-O antigen (2ug/slot) or the Op1 antigen (2 ug/slot) were absorbed using a slot blot apparatus. The results are shown in Figure 8, as follows:

Reactivity of 10H3 towards A4-O (upper panel):

Blot no:

1. Immunostain lacking the primary antibody (10H3) showed no reactivity with the blot, as expected.

2. 10H3 was very strongly reactive with its own antigen, A4-O.
3. Soluble A4-O antigen added to the mix caused inhibition of 10H3 towards A4-O.
4. Soluble Op1 added to the mix caused inhibition of 10H3 towards A4-O.
5. 10H3 was reactive towards the Op1 antigen.
6. Soluble Op1 added to the mix showed inhibition of 10H3 towards Op1.

The slot blots were quantified by densitometry and numerical values were obtained that indicated the extent of the reaction between 10H3 and antigens. These values are given below in Table I in which each numbered item refers to the blot number in Figure 8 and the description given above:

Table I: Optical Density of Reaction Between 10H3 and Either A4-O or Op1 Antigens in Blots of Figure 7

Slot Number:	1	2	3	4	5	6
OD Units:	0.03	0.70	0.31	0.18	0.16	0.07

Based upon the results presented in Figure 8 and Table I, applicants concluded that 10H3 is reactive with its own A4-O antigen as well as with the Op1 peptide.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE MIRIAM HOSPITAL

(ii) TITLE OF INVENTION: Composition and Method for in Vivo Imaging of Amyloid Deposits

(iii) NUMBER OF SEQUENCES: 13

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Foley & Lardner
(B) STREET: 3000 K Street, N.W., Suite 500
(C) CITY: Washington, D.C.
(E) COUNTRY: USA
(F) ZIP: 20007-5109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 27 May 1994
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: SAXE, Bernhard D.
(B) REGISTRATION NUMBER: 28,665
(C) REFERENCE/DOCKET NUMBER: 57548/103/MIHO

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (202)672-5300
(B) TELEFAX: (202)672-5399
(C) TELEX: 904136

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Gln Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
20 25

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
20 25 30

Gly Leu Met Val Gly Gly Val Val Ile Ala Thr
35 40

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
20 25 30

Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr
35 40 45

Leu Val Met Leu
50

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 99 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
20 25 30

Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr
35 40 45

Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val
50 55 60

Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys
65 70 75 80

Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln
85 90 95

Met Gln Asn

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp Ala Glu Phe Arg His
1 5 10 15

Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val Phe Phe Ala Glu
20 25 30

Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met Val Gly Gly
35 40 45

Val Val Ile Ala Thr
50

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Xaa at position 1 corresponds to 1 or more APP amino acids which are not adjacent to B/A4 in nature."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 45
(D) OTHER INFORMATION: /note= "Xaa at position 45 corresponds to 1 or more APP amino acids which are not adjacent to B/A4 in nature."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Xaa Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln
1 5 10 15

Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile
20 25 30

Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Xaa
35 40 45

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: A4-O

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Ser Ala
20 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: A4-P

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Gln Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
20 25

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: A4-B

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
20 25

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(B) CLONE: Op1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3353 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 147..2234

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
AGTTTCCTCG GCAGCGGTAG GCGAGAGCAC GCGGAGGAGC GTGCGCGGGG CCCCGGGAGA      60

CGGCGGCGGT GGCGGCGCGG GCAGAGCAAG GACGCGGCGG ATCCCACTCG CACAGCAGCG     120

CACTCGGTGC CCCGCGCAGG GTCGCG ATG CTG CCC GGT TTG GCA CTG CTC CTG       173
                             Met Leu Pro Gly Leu Ala Leu Leu Leu
                               1               5

CTG GCC GCC TGG ACG GCT CGG GCG CTG GAG GTA CCC ACT GAT GGT AAT       221
Leu Ala Ala Trp Thr Ala Arg Ala Leu Glu Val Pro Thr Asp Gly Asn
 10                  15                  20                  25

GCT GGC CTG CTG GCT GAA CCC CAG ATT GCC ATG TTC TGT GGC AGA CTG       269
Ala Gly Leu Leu Ala Glu Pro Gln Ile Ala Met Phe Cys Gly Arg Leu
                 30                  35                  40

AAC ATG CAC ATG AAT GTC CAG AAT GGG AAG TGG GAT TCA GAT CCA TCA       317
Asn Met His Met Asn Val Gln Asn Gly Lys Trp Asp Ser Asp Pro Ser
             45                  50                  55

GGG ACC AAA ACC TGC ATT GAT ACC AAG GAA GGC ATC CTG CAG TAT TGC       365
Gly Thr Lys Thr Cys Ile Asp Thr Lys Glu Gly Ile Leu Gln Tyr Cys
         60                  65                  70

CAA GAA GTC TAC CCT GAA CTG CAG ATC ACC AAT GTG GTA GAA GCC AAC       413
Gln Glu Val Tyr Pro Glu Leu Gln Ile Thr Asn Val Val Glu Ala Asn
     75                  80                  85

CAA CCA GTG ACC ATC CAG AAC TGG TGC AAG CGG GGC CGC AAG CAG TGC       461
Gln Pro Val Thr Ile Gln Asn Trp Cys Lys Arg Gly Arg Lys Gln Cys
 90                  95                 100                 105

AAG ACC CAT CCC CAC TTT GTG ATT CCC TAC CGC TGC TTA GTT GGT GAG       509
Lys Thr His Pro His Phe Val Ile Pro Tyr Arg Cys Leu Val Gly Glu
                110                 115                 120

TTT GTA AGT GAT GCC CTT CTC GTT CCT GAC AAG TGC AAA TTC TTA CAC       557
Phe Val Ser Asp Ala Leu Leu Val Pro Asp Lys Cys Lys Phe Leu His
            125                 130                 135

CAG GAG AGG ATG GAT GTT TGC GAA ACT CAT CTT CAC TGG CAC ACC GTC       605
Gln Glu Arg Met Asp Val Cys Glu Thr His Leu His Trp His Thr Val
        140                 145                 150

GCC AAA GAG ACA TGC AGT GAG AAG AGT ACC AAC TTG CAT GAC TAC GGC       653
Ala Lys Glu Thr Cys Ser Glu Lys Ser Thr Asn Leu His Asp Tyr Gly
    155                 160                 165

ATG TTG CTG CCC TGC GGA ATT GAC AAG TTC CGA GGG GTA GAG TTT GTG       701
Met Leu Leu Pro Cys Gly Ile Asp Lys Phe Arg Gly Val Glu Phe Val
170                 175                 180                 185

TGT TGC CCA CTG GCT GAA GAA AGT GAC AAT GTG GAT TCT GCT GAT GCG       749
```

```
Cys Cys Pro Leu Ala Glu Glu Ser Asp Asn Val Asp Ser Ala Asp Ala
                190                 195                 200

GAG GAG GAT GAC TCG GAT GTC TGG TGG GGC GGA GCA GAC ACA GAC TAT      797
Glu Glu Asp Asp Ser Asp Val Trp Trp Gly Gly Ala Asp Thr Asp Tyr
            205                 210                 215

GCA GAT GGG AGT GAA GAC AAA GTA GTA GAA GTA GCA GAG GAG GAA GAA      845
Ala Asp Gly Ser Glu Asp Lys Val Val Glu Val Ala Glu Glu Glu Glu
        220                 225                 230

GTG GCT GAG GTG GAA GAA GAA GAA GCC GAT GAT GAC GAG GAC GAT GAG      893
Val Ala Glu Val Glu Glu Glu Glu Ala Asp Asp Asp Glu Asp Asp Glu
    235                 240                 245

GAT GGT GAT GAG GTA GAG GAA GAG GCT GAG GAA CCC TAC GAA GAA GCC      941
Asp Gly Asp Glu Val Glu Glu Glu Ala Glu Glu Pro Tyr Glu Glu Ala
250                 255                 260                 265

ACA GAG AGA ACC ACC AGC ATT GCC ACC ACC ACC ACC ACC ACC ACA GAG      989
Thr Glu Arg Thr Thr Ser Ile Ala Thr Thr Thr Thr Thr Thr Thr Glu
                270                 275                 280

TCT GTG GAA GAG GTG GTT CGA GTT CCT ACA ACA GCA GCC AGT ACC CCT     1037
Ser Val Glu Glu Val Val Arg Val Pro Thr Thr Ala Ala Ser Thr Pro
            285                 290                 295

GAT GCC GTT GAC AAG TAT CTC GAG ACA CCT GGG GAT GAG AAT GAA CAT     1085
Asp Ala Val Asp Lys Tyr Leu Glu Thr Pro Gly Asp Glu Asn Glu His
        300                 305                 310

GCC CAT TTC CAG AAA GCC AAA GAG AGG CTT GAG GCC AAG CAC CGA GAG     1133
Ala His Phe Gln Lys Ala Lys Glu Arg Leu Glu Ala Lys His Arg Glu
    315                 320                 325

AGA ATG TCC CAG GTC ATG AGA GAA TGG GAA GAG GCA GAA CGT CAA GCA     1181
Arg Met Ser Gln Val Met Arg Glu Trp Glu Glu Ala Glu Arg Gln Ala
330                 335                 340                 345

AAG AAC TTG CCT AAA GCT GAT AAG AAG GCA GTT ATC CAG CAT TTC CAG     1229
Lys Asn Leu Pro Lys Ala Asp Lys Lys Ala Val Ile Gln His Phe Gln
                350                 355                 360

GAG AAA GTG GAA TCT TTG GAA CAG GAA GCA GCC AAC GAG AGA CAG CAG     1277
Glu Lys Val Glu Ser Leu Glu Gln Glu Ala Ala Asn Glu Arg Gln Gln
            365                 370                 375

CTG GTG GAG ACA CAC ATG GCC AGA GTG GAA GCC ATG CTC AAT GAC CGC     1325
Leu Val Glu Thr His Met Ala Arg Val Glu Ala Met Leu Asn Asp Arg
        380                 385                 390

CGC CGC CTG GCC CTG GAG AAC TAC ATC ACC GCT CTG CAG GCT GTT CCT     1373
Arg Arg Leu Ala Leu Glu Asn Tyr Ile Thr Ala Leu Gln Ala Val Pro
    395                 400                 405

CCT CGG CCT CGT CAC GTG TTC AAT ATG CTA AAG AAG TAT GTC CGC GCA     1421
Pro Arg Pro Arg His Val Phe Asn Met Leu Lys Lys Tyr Val Arg Ala
410                 415                 420                 425

GAA CAG AAG GAC AGA CAG CAC ACC CTA AAG CAT TTC GAG CAT GTG CGC     1469
Glu Gln Lys Asp Arg Gln His Thr Leu Lys His Phe Glu His Val Arg
                430                 435                 440

ATG GTG GAT CCC AAG AAA GCC GCT CAG ATC CGG TCC CAG GTT ATG ACA     1517
Met Val Asp Pro Lys Lys Ala Ala Gln Ile Arg Ser Gln Val Met Thr
            445                 450                 455
```

```
CAC CTC CGT GTG ATT TAT GAG CGC ATG AAT CAG TCT CTC TCC CTG CTC      1565
His Leu Arg Val Ile Tyr Glu Arg Met Asn Gln Ser Leu Ser Leu Leu
        460                 465                 470

TAC AAC GTG CCT GCA GTG GCC GAG GAG ATT CAG GAT GAA GTT GAT GAG      1613
Tyr Asn Val Pro Ala Val Ala Glu Glu Ile Gln Asp Glu Val Asp Glu
    475                 480                 485

CTG CTT CAG AAA GAG CAA AAC TAT TCA GAT GAC GTC TTG GCC AAC ATG      1661
Leu Leu Gln Lys Glu Gln Asn Tyr Ser Asp Asp Val Leu Ala Asn Met
490                 495                 500                 505

ATT AGT GAA CCA AGG ATC AGT TAC GGA AAC GAT GCT CTC ATG CCA TCT      1709
Ile Ser Glu Pro Arg Ile Ser Tyr Gly Asn Asp Ala Leu Met Pro Ser
                510                 515                 520

TTG ACC GAA ACG AAA ACC ACC GTG GAG CTC CTT CCC GTG AAT GGA GAG      1757
Leu Thr Glu Thr Lys Thr Thr Val Glu Leu Leu Pro Val Asn Gly Glu
            525                 530                 535

TTC AGC CTG GAC GAT CTC CAG CCG TGG CAT TCT TTT GGG GCT GAC TCT      1805
Phe Ser Leu Asp Asp Leu Gln Pro Trp His Ser Phe Gly Ala Asp Ser
        540                 545                 550

GTG CCA GCC AAC ACA GAA AAC GAA GTT GAG CCT GTT GAT GCC CGC CCT      1853
Val Pro Ala Asn Thr Glu Asn Glu Val Glu Pro Val Asp Ala Arg Pro
    555                 560                 565

GCT GCC GAC CGA GGA CTG ACC ACT CGA CCA GGT TCT GGG TTG ACA AAT      1901
Ala Ala Asp Arg Gly Leu Thr Thr Arg Pro Gly Ser Gly Leu Thr Asn
570                 575                 580                 585

ATC AAG ACG GAG GAG ATC TCT GAA GTG AAG ATG GAT GCA GAA TTC CGA      1949
Ile Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp Ala Glu Phe Arg
                590                 595                 600

CAT GAC TCA GGA TAT GAA GTT CAT CAT CAA AAA TTG GTG TTC TTT GCA      1997
His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val Phe Phe Ala
            605                 610                 615

GAA GAT GTG GGT TCA AAC AAA GGT GCA ATC ATT GGA CTC ATG GTG GGC      2045
Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met Val Gly
        620                 625                 630

GGT GTT GTC ATA GCG ACA GTG ATC GTC ATC ACC TTG GTG ATG CTG AAG      2093
Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu Val Met Leu Lys
    635                 640                 645

AAG AAA CAG TAC ACA TCC ATT CAT CAT GGT GTG GTG GAG GTT GAC GCC      2141
Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val Glu Val Asp Ala
650                 655                 660                 665

GCT GTC ACC CCA GAG GAG CGC CAC CTG TCC AAG ATG CAG CAG AAC GGC      2189
Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met Gln Gln Asn Gly
                670                 675                 680

TAC GAA AAT CCA ACC TAC AAG TTC TTT GAG CAG ATG CAG AAC TAGACCCCCG   2241
Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln Met Gln Asn
            685                 690                 695

CCACAGCAGC CTCTGAAGTT GGACAGCAAA ACCATTGCTT CACTACCCAT CGGTGTCCAT   2301

TTATAGAATA ATGTGGGAAG AAACAAACCC GTTTTATGAT TTACTCATTA TCGCCTTTTG   2361

ACAGCTGTGC TGTAACACAA GTAGATGCCT GAACTTGAAT TAATCCACAC ATCAGTAATG   2421

TATTCTATCT CTCTTTACAT TTTGGTCTCT ATACTACATT ATTAATGGGT TTTGTGTACT   2481
```

GTAAAGAATT TAGCTGTATC AAACTAGTGC ATGAATAGAT TCTCTCCTGA TTATTTATCA 2541

CATAGCCCCT TAGCCAGTTG TATATTATTC TTGTGGTTTG TGACCCAATT AAGTCCTACT 2601

TTACATATGC TTTAAGAATC GATGGGGGAT GCTTCATGTG AACGTGGGAG TTCAGCTGCT 2661

TCTCTTGCCT AAGTATTCCT TTCCTGATCA CTATGCATTT TAAAGTTAAA CATTTTTAAG 2721

TATTTCAGAT GCTTTAGAGA GATTTTTTTT CCATGACTGC ATTTTACTGT ACAGATTGCT 2781

GCTTCTGCTA TATTTGTGAT ATAGGAATTA AGAGGATACA CACGTTTGTT TCTTCGTGCC 2841

TGTTTTATGT GCACACATTA GGCATTGAGA CTTCAAGCTT TTCTTTTTTT GTCCACGTAT 2901

CTTTGGGTCT TTGATAAAGA AAAGAATCCC TGTTCATTGT AAGCACTTTT ACGGGGCGGG 2961

TGGGGAGGGG TGCTCTGCTG GTCTTCAATT ACCAAGAATT CTCCAAAACA ATTTTCTGCA 3021

GGATGATTGT ACAGAATCAT TGCTTATGAC ATGATCGCTT TCTACACTGT ATTACATAAA 3081

TAAATTAAAT AAAATAACCC CGGGCAAGAC TTTTCTTTGA AGGATGACTA CAGACATTAA 3141

ATAATCGAAG TAATTTTGGG TGGGGAGAAG AGGCAGATTC AATTTTCTTT AACCAGTCTG 3201

AAGTTTCATT TATGATACAA AAGAAGATGA AAATGGAAGT GGCAATATAA GGGGATGAGG 3261

AAGGCATGCC TGGACAAACC CTTCTTTTAA GATGTGTCTT CAATTTGTAT AAAATGGTGT 3321

TTTCATGTAA ATAAATACAT TCTTGGAGGA GC 3353

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 695 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
 1               5                  10                  15

Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
            20                  25                  30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
        35                  40                  45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
    50                  55                  60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65                  70                  75                  80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
                85                  90                  95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
            100                 105                 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
        115                 120                 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
```

```
    130                 135                 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145                 150                 155                 160

Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165                 170                 175

Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
            180                 185                 190

Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
        195                 200                 205

Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
    210                 215                 220

Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225                 230                 235                 240

Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
                245                 250                 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
            260                 265                 270

Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
        275                 280                 285

Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
    290                 295                 300

Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305                 310                 315                 320

Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
                325                 330                 335

Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
            340                 345                 350

Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
        355                 360                 365

Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
    370                 375                 380

Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385                 390                 395                 400

Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
                405                 410                 415

Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
            420                 425                 430

Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
        435                 440                 445

Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
    450                 455                 460

Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
465                 470                 475                 480

Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
                485                 490                 495
```

```
Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
            500                 505                 510

Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
        515                 520                 525

Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
    530                 535                 540

Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
545                 550                 555                 560

Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
                565                 570                 575

Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
            580                 585                 590

Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
        595                 600                 605

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
    610                 615                 620

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625                 630                 635                 640

Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
                645                 650                 655

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
            660                 665                 670

His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
        675                 680                 685

Phe Phe Glu Gln Met Gln Asn
    690                 695
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 11
(D) OTHER INFORMATION: /note= ""Xaa at Position 11 is either Glu or Gln.""

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 27
(D) OTHER INFORMATION: /note= ""Xaa at postion 27 is either Ser or Asn.""

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 28
(D) OTHER INFORMATION: /note= ""Xaa at position 28 is either Ala of Lys.""

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Xaa Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Xaa Xaa
20 25

What Is Claimed Is:

1. An amyloid binding composition for *in vivo* imaging of amyloid deposits comprising:

(a) a labeled amyloid protein or variant thereof that binds to amyloid deposits *in vivo*; and

(b) a pharmaceutically acceptable carrier.

2. The composition of claim 1, wherein said amyloid protein is ß-amyloid polypeptide or a variant thereof.

3. The composition of claim 2, wherein said ß-amyloid polypeptide variant has the following amino acid sequence (SEQ ID NO:13):

N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-X-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Y1-Y2-COOH;

wherein X is either Glu or Gln; Y1 is either Ser or Asn; and Y2 is either Ala or Lys.

4. The composition of claim 3, wherein said ß-amyloid polypeptide variant is selected from the group consisting of (1) a variant wherein when X is Glu, Y1 is Ser and Y2 is Ala, (2) a variant wherein when X is Glu, Y1 is Asn and Y2 is Lys, and (3) a variant wherein when X is Gln, Y1 is Asn and Y2 is Lys.

5. The composition of claim 2, wherein said ß-amyloid polypeptide or variant thereof has an amino acid sequence selected from the following group of amino acid sequences:

(A) (SEQ ID NO:2) Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr;

(B) (SEQ ID NO:3) Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-

Gly-Gly-Val-Val-Ile-Ala-Thr-Val-Ile-Val-Ile-Thr-Leu-Val-Met-Leu;

(C)(SEQ ID NO:4) Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr-Val-Ile-Val-Ile-Thr-Leu-Val-Met-Leu-Lys-Lys-Lys-Gln-Tyr-Thr-Ser-Ile-His-His-Gly-Val-Val-Glu-Val-Asp-Ala-Ala-Val-Thr-Pro-Glu-Glu-Arg-His-Leu-Ser-Lys-Met-Gln-Gln-Asn-Gly-Tyr-Glu-Asn-Pro-Thr-Tyr-Lys-Phe-Phe-Glu-Gln-Met-Gln-Asn;

(D)(SEQ ID NO:5) Lys-Thr-Glu-Glu-Ile-Ser-Glu-Val-Lys-Met-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr;

(E)(SEQ ID NO:6) X-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr-Y,

wherein X and Y are one or more APP amino acids which are not ajacent to β/A4 in the nature;

and

(F) any fragment of (A)-(E), wherein said fragment is large enough to bind amyloid deposit *in vivo*.

6. The composition of claim 1, wherein said labeled amyloid protein is radiolabeled amyloid protein.

7. The composition of claim 1, wherein said radiolabeled amyloid protein is Technetium 99m-labeled amyloid protein.

8. An *in vivo* method for detecting amyloid deposits in a subject comprising the steps of

(a) administering to a subject a detectable quantity of an amyloid binding composition comprising a labeled amyloid protein or variant thereof and a pharmaceutically acceptable carrier; and

(b) detecting the binding of the labeled protein or variant thereof to the amyloid deposit.

9. The method of claim 8, wherein said amyloid protein is the ß-amyloid polypeptide or variant thereof.

10. The method of claim 8, wherein said amyloid protein is radiolabeled.

11. The method of claim 10, wherein said detecting involves radioactive imaging.

12. The method of claim 8, wherein said administering is selected from the group consisting of intravenous injection, intraventricular injection and a combination of both intravenous and intraventricular injection.

13. The method of claim 8, wherein said amyloid deposits are located in the brain of a subject.

14. A method of diagnosing an amyloidosis-associated disease by detecting amyloid deposits in a subject suspected of having amyloid deposits, said method comprising the steps of:

(a) administering to a subject a detectable quantity of an amyloid binding composition comprising a labeled amyloid protein or variant thereof and a pharmaceutically acceptable carrier; and

(b) detecting the binding of said labeled protein to said amyloid deposit.

15. The method of claim 14, wherein said amyloidosis-associated disease is selected from the group consisting of Alzheimer's Disease and Down Syndrome.

FIG. 1

Clinical Association	Notation	Protein Type
ACQUIRED SYSTEMIC AMYLOIDOSIS		
Immunoglobulin light-chain (primary), Multiple myeloma	AL	Light chain, type subtype
Reactive (secondary)	AA	Protein A
Hemodialysis amyloidosis	AH	β_2 microglobulin
HEREDOFAMILIAL		
Polyneuropathy	AF	Prealbumin, variant
Familial Mediterranean fever	AA	Protein A
ORGAN-LIMITED		
Hereditary Icelandic Congophilic angiopathy	ACv_C	Cystatin C, variant
Alzheimer's disease: vessels and plaques	$ACv\beta 1$	β protein
Senile cardiac	$ACp\beta$	
LOCALIZED ENDOCRINE		
Pancreatic islet	AE_f	Islet amyloid protein [IAP]
Medullary thyroid carcinoma	AE_t	Precalcitonin

FIG. 2

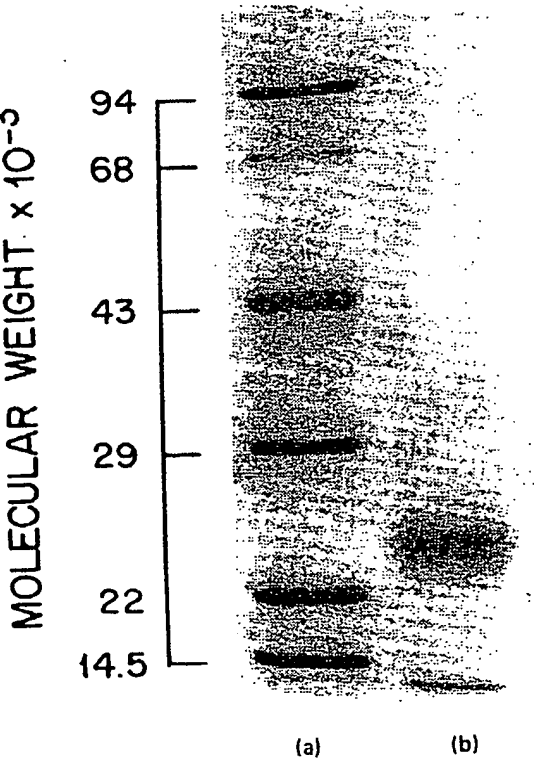


FIG. 3

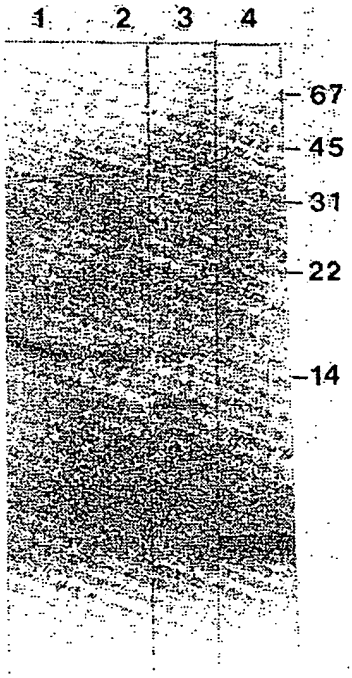


FIG. 4

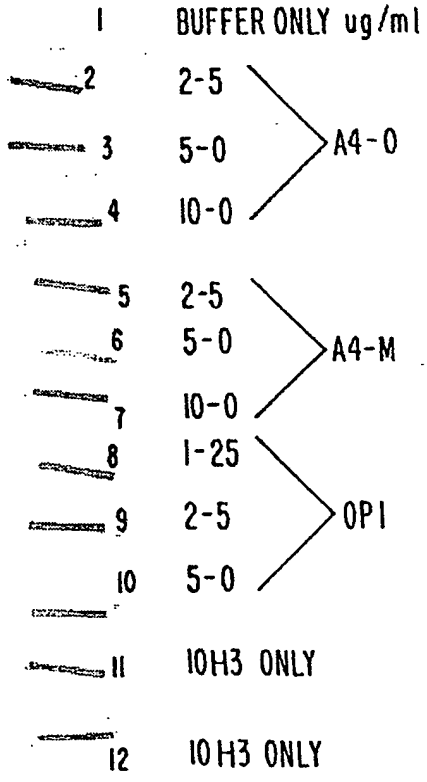

1
BUFFER ONLY ug/ml
2
2-5
3
5-0
4
10-0
A4-0
5
2-5
6
5-0
7
10-0
A4-M
8
1-25
9
2-5
10
5-0
OPI
11
10H3 ONLY
12
10H3 ONLY

FIG. 5

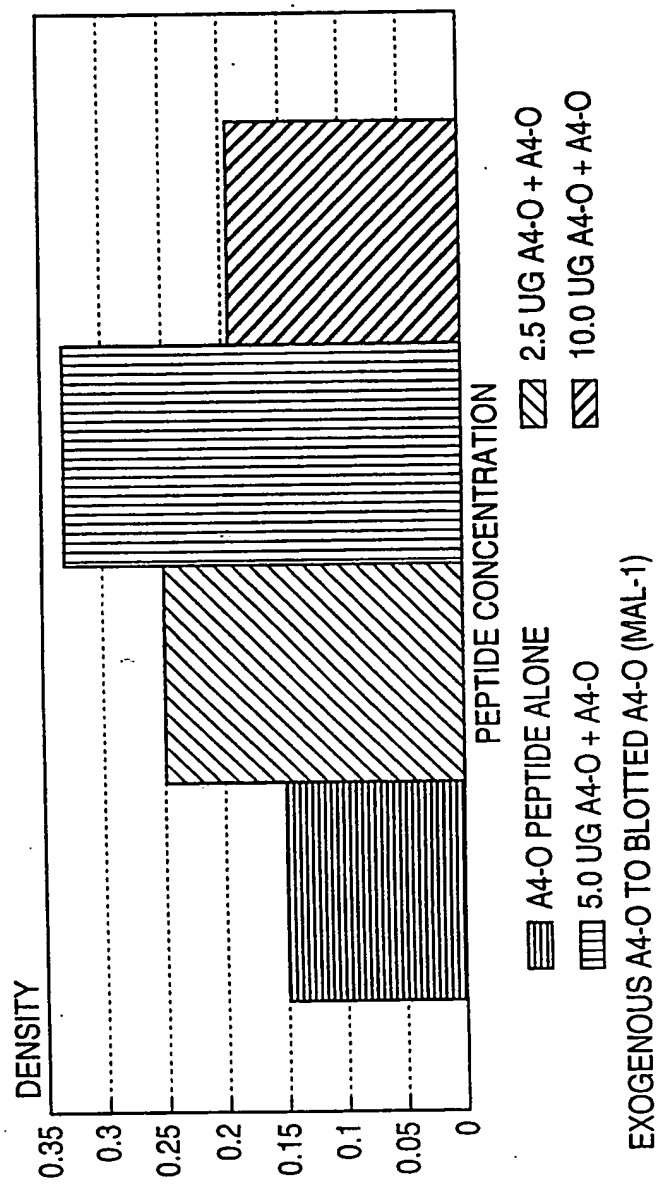

A4-O IMMUNOBLOT
AGGREGATION OF A4-O + A4-O
DENSITY
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
PEPTIDE CONCENTRATION
A4-O PEPTIDE ALONE
5.0 UG A4-O + A4-O
2.5 UG A4-O + A4-O
10.0 UG A4-O + A4-O
EXOGENOUS A4-O TO BLOTTED A4-O (MAL-1)

FIG. 6

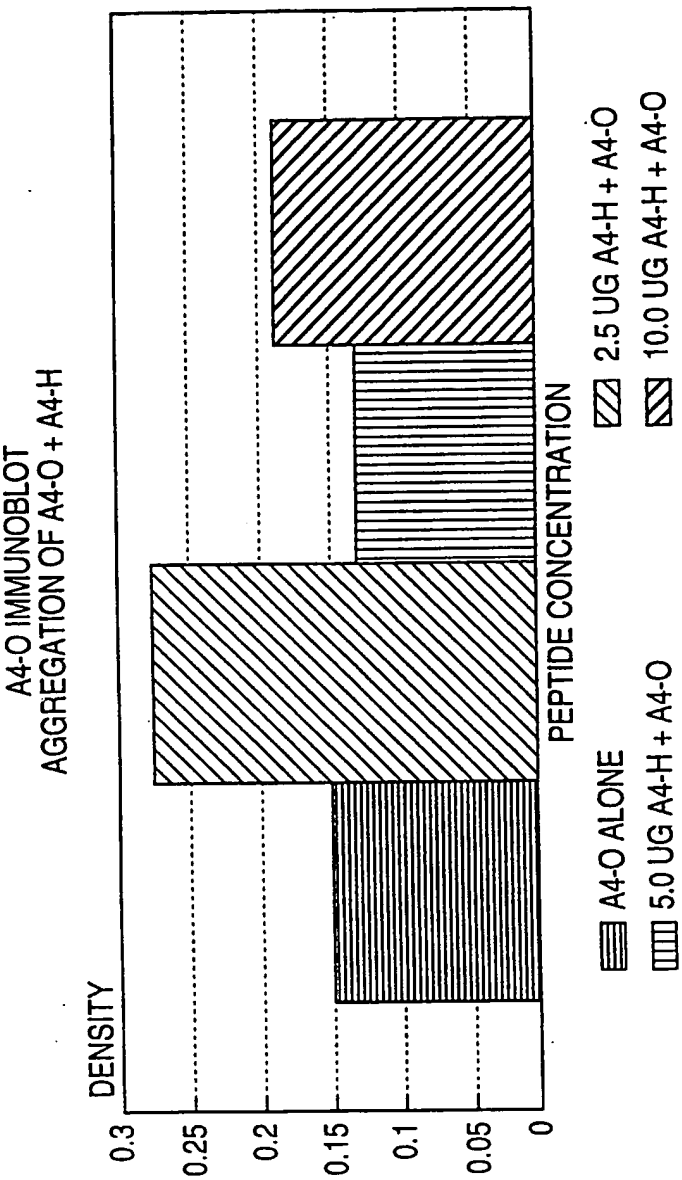

A4-O IMMUNOBLOT
AGGREGATION OF A4-O + A4-H
DENSITY
0.3
0.25
0.2
0.15
0.1
0.05
0
PEPTIDE CONCENTRATION
A4-O ALONE
5.0 UG A4-H + A4-O
2.5 UG A4-H + A4-O
10.0 UG A4-H + A4-O
EXOGENOUS A4-H TO BLOTTED A4-O (MAL-2)

FIG. 7

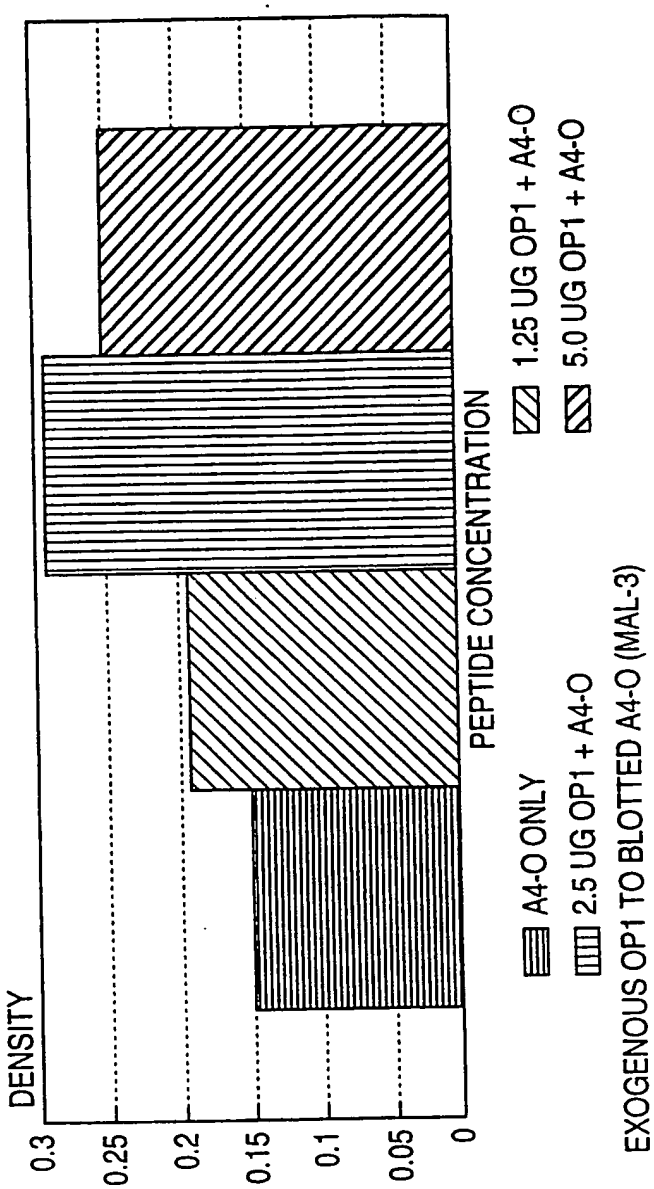

A4-O IMMUNOBLOT
AGGREGATION OF A4-O + OP1
DENSITY
0.3
0.25
0.2
0.15
0.1
0.05
0
PEPTIDE CONCENTRATION
A4-O ONLY
2.5 UG OP1 + A4-O
1.25 UG OP1 + A4-O
5.0 UG OP1 + A4-O
EXOGENOUS OP1 TO BLOTTED A4-O (MAL-3)

FIG. 8

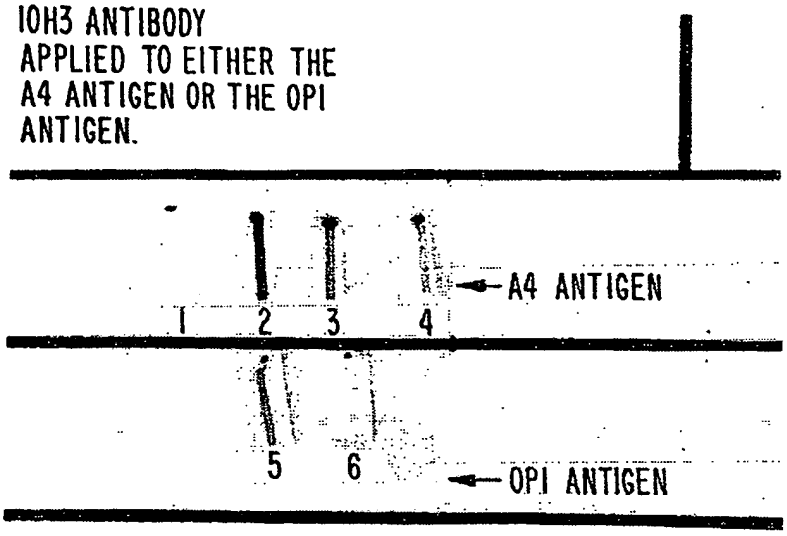

10H3 ANTIBODY
APPLIED TO EITHER THE
A4 ANTIGEN OR THE OPI
ANTIGEN.
A4 ANTIGEN
1
2
3
4
5
6
OPI ANTIGEN

FIG. 9A


```
                                                    AGTTTCCTCGGCAGCGGTAGGCGAGA -121

2 GCACGCGGAGGAGCGTGCGCGGGGCCCCGGGAGACGGCGGCGGTGGCGGCGCGGGCAGAG -61

3 CAAGGACGCGGCGGATCCCACTCGCACAGCAGCGCACTCGGTGCCCCGCGCAGGGTCGCG -1

4 ATGCTGCCCGGTTTGGCACTGCTCCTGCTGGCCGCCTGGACGGCTCGGGCGCTGGAGGTA 60
  M  L  P  G  L  A  L  L  L  L  A  A  W  T  A  R  A  L  E  V
  1                          10                             20

5 CCCACTGATGGTAATGCTGGCCTGCTGGCTGAACCCCAGATTGCCATGTTCTGTGGCAGA 120
  P  T  D  G  N  A  G  L  L  A  E  P  Q  I  A  M  F  C  G  R
                             30                             40

6 CTGAACATGCACATGAATGTCCAGAATGGGAAGTGGGATTCAGATCCATCAGGGACCAAA 180
  L  N  M  H  M  N  V  Q  N  G  K  W  D  S  D  P  S  G  T  K
                             50                             60

7 ACCTGCATTGATACCAAGGAAGGCATCCTGCAGTATTGCCAAGAAGTCTACCCTGAACTG 240
  T  C  I  D  T  K  E  G  I  L  Q  Y  C  Q  E  V  Y  P  E  L
                             70                             80

8 CAGATCACCAATGTGGTAGAAGCCAACCAACCAGTGACCATCCAGAACTGGTGCAAGCGG 300
  Q  I  T  N  V  V  E  A  N  Q  P  V  T  I  Q  N  W  C  K  R
                             90                            100

9 GGCCGCAAGCAGTGCAAGACCCATCCCCACTTTGTGATTCCCTACCGCTGCTTAGTTGGT 360
  G  R  K  Q  C  K  T  H  P  H  F  V  I  P  Y  R  C  L  V  G
                            110                            120

10 GAGTTTGTAAGTGATGCCCTTCTCGTTCCTGACAAGTGCAAATTCTTACACCAGGAGAGG 420
   E  F  V  S  D  A  L  L  V  P  D  K  C  K  F  L  H  Q  E  R
                            130                            140

11 ATGGATGTTTGCGAAACTCATCTTCACTGGCACACCGTCGCCAAAGAGACATGCAGTGAG 480
   M  D  V  C  E  T  H  L  H  W  H  T  V  A  K  E  T  C  S  E
                            150                            160

12 AAGAGTACCAACTTGCATGACTACGGCATGTTGCTGCCCTGCGGAATTGACAAGTTCCGA 540
   K  S  T  N  L  H  D  Y  G  M  L  L  P  C  G  I  D  K  F  R
                            170                            180

13 GGGGTAGAGTTTGTGTGTTGCCCACTGGCTGAAGAAAGTGACAATGTGGATTCTGCTGAT 600
   G  V  E  F  V  C  C  P  L  A  E  E  S  D  N  V  D  S  A  D
                            190                            200

14 GCGGAGGAGGATGACTCGGATGTCTGGTGGGGCGGAGCAGACACAGACTATGCAGATGGG 660
   A  E  E  D  D  S  D  V  W  W  G  G  A  D  T  D  Y  A  D  G
                            210                            220

15 AGTGAAGACAAAGTAGTAGAAGTAGCAGAGGAGGAAGAAGTGGCTGAGGTGGAAGAAGAA 720
   S  E  D  K  V  V  E  V  A  E  E  E  E  V  A  E  V  E  E  E
                            230                            240

16 GAAGCCGATGATGACGAGGACGATGAGGATGGTGATGAGGTAGAGGAAGAGGCTGAGGAA 780
   E  A  D  D  D  E  D  D  E  D  G  D  E  V  E  E  E  A  E  E
                            250                            260

17 CCCTACGAAGAAGCCACAGAGAGAACCACCAGCATTGCCACCACCACCACCACCACCACA 840
   P  Y  E  E  A  T  E  R  T  T  S  I  A  T  T  T  T  T  T  T
                            270                            280

18 GAGTCTGTGGAAGAGGTGGTTCGAGTTCCTACAACAGCAGCCAGTACCCCTGATGCCGTT 900
   E  S  V  E  E  V  V  R  V  P  T  T  A  A  S  T  P  D  A  V
                            290                            300

19 GACAAGTATCTCGAGACACCTGGGGATGAGAATGAACATGCCCATTTCCAGAAAGCCAAA 960
   D  K  Y  L  E  T  P  G  D  E  N  E  H  A  H  F  Q  K  A  K
                            310                            320

20 GAGAGGCTTGAGGCCAAGCACCGAGAGAGAATGTCCCAGGTCATGAGAGAATGGGAAGAG 1020
   E  R  L  E  A  K  H  R  E  R  M  S  Q  V  M  R  E  W  E  E
                            330                            340

21 GCAGAACGTCAAGCAAAGAACTTGCCTAAAGCTGATAAGAAGGCAGTTATCCAGCATTTC 1080
   A  E  R  Q  A  K  N  L  P  K  A  D  K  K  A  V  I  Q  H  F
                            350                            360

22 CAGGAGAAAGTGGAATCTTTGGAACAGGAAGCAGCCAACGAGAGACAGCAGCTGGTGGAG 1140
   Q  E  K  V  E  S  L  E  Q  E  A  A  N  E  R  Q  Q  L  V  E
                            370                            380

23 ACACACATGGCCAGAGTGGAAGCCATGCTCAATGACCGCCGCCGCCTGGCCCTGGAGAAC 1200
   T  H  M  A  R  V  E  A  M  L  N  D  R  R  R  L  A  L  E  N
                            390                            400

24 TACATCACCGCTCTGCAGGCTGTTCCTCCTCGGCCTCGTCACGTGTTCAATATGCTAAAG 1260
   Y  I  T  A  L  Q  A  V  P  P  R  P  R  H  V  F  N  M  L  K
                            410                            420

25 AAGTATGTCCGCGCAGAACAGAAGGACAGACAGCACACCCTAAAGCATTTCGAGCATGTG 1320
   C  Y  V  R  A  E  Q  K  D  R  Q  H  T  L  K  H  F  E  H  V
                            430                            440
```

FIG. 9B

```
26 CGCATGGTGGATCCCAAGAAAGCCGCTCAGATCCGGTCCCAGGTTATGACACACCTCCGT 1380
   R  M  V  D  P  K  K  A  A  Q  I  R  S  Q  V  M  T  M  L  R
                              450                             460

27 GTGATTTATGAGCGCATGAATCAGTCTCTCTCCCTGCTCTACAACGTGCCTGCAGTGGCC 1440
   V  I  Y  E  R  M  N  Q  S  L  S  L  L  Y  N  V  P  A  V  A
                              470                             480

28 GAGGAGATTCAGGATGAAGTTGATGAGCTGCTTCAGAAAGAGCAAAACTATTCAGATGAC 1500
   E  E  I  Q  D  E  V  D  E  L  L  Q  K  E  Q  N  Y  S  D  D
                              490                             500

29 GTCTTGGCCAACATGATTAGTGAACCAAGGATCAGTTACGGAAACGATGCTCTCATGCCA 1560
   V  L  A  N  M  I  S  E  P  R  I  S  Y  G  N  D  A  L  M  P
                              510                             520

30 TCTTTGACCGAAACGAAAACCACCGTGGAGCTCCTTCCCGTGAATGGAGAGTTCAGCCTG 1620
   S  L  T  E  T  K  T  T  V  E  L  L  P  V  N  G  E  F  S  L
                              530                             540

31 GACGATCTCCAGCCGTGGCATTCTTTTGGGGCTGACTCTGTGCCAGCCAACACAGAAAAC 1680
   D  D  L  Q  P  W  H  S  F  G  A  D  S  V  P  A  N  T  E  N
                              550                             560

32 GAAGTTGAGCCTGTTGATGCCCGCCCTGCTGCCGACCGAGGACTGACCACTCGACCAGGT 1740
   E  V  E  P  V  D  A  R  P  A  A  D  R  G  L  T  T  R  P  G
                              570                             580

33 TCTGGGTTGACAAATATCAAGACGGAGGAGATCTCTGAAGTGAAGATGGATGCAGAATTC 1800
   S  G  L  T  N  I  K  T  E  E  I  S  E  V  K  M  D  A  E  F
                              590                             600

34 CGACATGACTCAGGATATGAAGTTCATCATCAAAAATTGGTGTTCTTTGCAGAAGATGTG 1860
   R  H  D  S  G  Y  E  V  H  H  Q  K  L  V  F  F  A  E  D  V
                              610                             620

35 GGTTCAAACAAAGGTGCAATCATTGGACTCATGGTGGGCGGTGTTGTCATAGCGACAGTG 1920
   G  S  N  K  G  A  I  I  G  L  M  V  G  G  V  V  I  A  T  V
                              630                             640

36 ATCGTCATCACCTTGGTGATGCTGAAGAAGAAACAGTACACATCCATTCATCATGGTGTG 1980
   I  V  I  T  L  V  M  L  K  K  K  Q  Y  T  S  I  H  H  G  V
                              650                             660

37 GTGGAGGTTGACGCCGCTGTCACCCCAGAGGAGCGCCACCTGTCCAAGATGCAGCAGAAC 2040
   V  E  V  D  A  A  V  T  P  E  E  R  H  L  S  K  M  Q  Q  N
                              670                             680

38 GGCTACGAAAATCCAACCTACAAGTTCTTTGAGCAGATGCAGAACTAGACCCCCGCCACA 2100
   G  Y  E  N  P  T  Y  K  F  F  E  Q  M  Q  N  *
                              690

39 GCAGCCTCTGAAGTTGGACAGCAAAACCATTGCTTCACTACCCATCGGTGTCCATTTATA 2160
40 GAATAATGTGGGAAGAAACAAACCCGTTTTATGATTTACTCATTATCGCCTTTTGACAGC 2220
41 TGTGCTGTAACACAAGTAGATGCCTGAACTTGAATTAATCCACACATCAGTAATGTATTC 2280
42 TATCTCTCTTTACATTTTGGTCTCTATACTACATTATTAATGGGTTTTGTGTACTGTAAA 2340
43 GAATTTAGCTGTATCAAACTAGTGCATGAATAGATTCTCTCCTGATTATTTATCACATAG 2400
44 CCCCTTAGCCAGTTGTATATTATTCTTGTGGTTTGTGACCCAATTAAGTCCTACTTTACA 2460
45 TATGCTTTAAGAATCGATGGGGGATGCTTCATGTGAACGTGGGAGTTCAGCTGCTTCTCT 2520
46 TGCCTAAGTATTCCTTTCCTGATCACTATGCATTTTAAAGTTAAACATTTTTAAGTATTT 2580
47 CAGATGCTTTAGAGAGATTTTTTTTCCATGACTGCATTTTACTGTACAGATTGCTGCTTC 2640
48 TGCTATATTTGTGATATAGGAATTAAGAGGATACACACGTTTGTTTCTTCGTGCCTGTTT 2700
49 TATGTGCACACATTAGGCATTGAGACTTCAAGCTTTTCTTTTTTTGTCCACGTATCTTTG 2760
50 GGTCTTTGATAAAGAAAAGAATCCCTGTTCATTGTAAGCACTTTTACGGGGCGGGTGGGG 2820
51 AGGGGTGCTCTGCTGGTCTTCAATTACCAAGAATTCTCCAAAACAATTTTCTGCAGGATG 2880
52 ATTGTACAGAATCATTGCTTATGACATGATCGCTTTCTACACTGTATTACATAAATAAAT 2940
53 TAAATAAAATAACCCCGGGCAAGACTTTTCTTTGAAGGATGACTACAGACATTAAATAAT 3000
54 CGAAGTAATTTTGGGTGGGGAGAAGAGGCAGATTCAATTTTCTTTAACCAGTCTGAAGTT 3060
55 TCATTTATGATACAAAAGAAGATGAAAATGGAAGTGGCAATATAAGGGGATGAGGAAGGC 3120
56 ATGCCTGGACAAACCCTTCTTTTAAGATGTGTCTTCAATTTGTATAAAATGGTGTTTTCA 3180
57 TGTAAATAAATACATTCTTGGAGGAGC-poly(A)tall
```

INTERNATIONAL SEARCH REPORT

I .ational application No.
PCT/US94/05809

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :G01N 33/367; A61K 49/00, 43/00
US CL :435/7.21; 424/1.11, 1.57, 9; 514/2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1, 7.2, 7.21; 424/1.11, 1.57, 1.69, 9; 514/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Medline, EMBASE, BIOSIS, CA Search, WPO, APS, IntelliGenetics

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO, A, 93/04194 (MAGGIO ET AL) 04 March 1993, see entire document, especially page 4, lines 8-15, page 8, lines 14-17, page 12, lines 15-39 and page 14, lines 26-36.	1, 2, 6, 7, 8-15 3-5
Y	Proceedings of the National Academy of Science USA, Volume 87, issued June 1992, Maggio et al, "Reversible in vitro growth of Alzheimer disease beta-amyloid plaques by deposition of labeled amyloid peptide", pages 5462-5466, especially see Abstract on page 5462.	1-15

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
24 AUGUST 1994	SEP 06 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CAROL E. BIDWELL Jill Warden for Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05809

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Society for Neuroscience Abstracts from the 21st Annual Meeting, Volume 17, Number 1-2, issued Nov. 1991, Mantyh e tla, "Distribution and Characterization of Amyloid Beta Protein Deposition in Normal Human and Alzheimer's Diseased Cerebral Cortx Using 125I-BAP as the Radioligand", page 912, Abstract 364.4, see entire abstract.	1, 2, 6, 7 3-5, 8-15
Y	Nature, Volume 325, issued 19 February 1987, Kang et al "The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor", pages 733-735, especially see Fig. 2.	1-15
A	Freeman et al, editors, Physician Desk Reference For Radiology and Nuclear Medicine, published 1977 by Medical Economics Company, Oradell, N.J., pages 39-43.	1-15
A	Proceedings of the National Academy of Science USA, Volume 88, issued August 1991, Kowall et al, "An in vivo model for the neurodegenerative effects of beta amyloid and protection by substance P", pages 7247-7251.	1-15
A	Neurobiology of Aging, Volume 13, Number 5, issued 1992, May et al, "Beta-Amyloid Peptide In Vitro Toxicity: Lot-to-Lit Variability", pages 605-607.	1-15